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Habitat and prey preferences of the two predatory bugs *Anthocoris nemorum* (L) and *A. nemoralis* (Fabricius) (Anthocoridae: Hemiptera-Heteroptera)

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Running title: Habitat and prey preferences of two anthocorids

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Abstract

The annual occurrence and distribution of the predatory bugs *Anthocoris nemorum* and *A. nemoralis* between apple, pear and herbal vegetation, was assessed in three apple orchards in 2001, and in two apple and two pear orchards in 2002. In the laboratory anthocorid prey preference was assessed in two-choice experiments with key pests of apple and pear including pear psyllid, apple psyllid, green apple aphid, rosy apple aphid and red spider mites. Anthocorid predation behaviour towards pear psyllids was assessed. Anthocorids were the dominant early season predatory bugs. *Anthocoris nemorum* dominated in apple, while *A. nemoralis* dominated in pear. *A. nemorum* was also common in herbal vegetation, especially in midsummer. Anthocorid numbers were correlated with numbers of collembola, psyllids and aphids in apple, and with numbers of psyllids in pear. *A. nemoralis* preferred pear psyllid to green apple aphid, while *A. nemorum* preferred green apple aphid. Both species preferred psyllids to spider mites. The success rate of *A. nemoralis* in attacking pear psyllids was higher than that of *A. nemorum*. *A. nemorum* had two generations in both 2001 and 2002 proving that it can be bivoltine under Danish climate conditions. The midsummer move by *A. nemorum* from trees to herbal vegetation suggests that herbal vegetation may maintain *A. nemorum* in orchards at times of low prey numbers in the trees. Habitat and prey preference trials both identify *A. nemorum* as a biological control agent of special importance in apple, whereas *A. nemoralis* is of importance in pear.

Key words: predation; voltinism; orchard; psyllids; aphids; preference; behaviour; *Anthocoris nemorum*; *Anthocoris nemoralis*; *Cacopsylla mali*; *Cacopsylla pyri*; *Aphis pomi*; *Dysaphis plantaginea*; *Panonychus ulmi*

1. Introduction

Two of the most abundant predators in apple and pear orchards of Europe are *Anthocoris nemorum* (L.) and *A. nemoralis* (Fabricius) (Anthocoridae: Hemiptera-Heteroptera) (Skanland 1981; Solomon 1982). Both are polyphagous predators that prey on aphids, mites, psyllids, and lepidopteran eggs and young larvae, all of which they are considered to play an important role in controlling (Anderson, 1962a; Anderson, 1962b; Collyer, 1967; Hill, 1957; Solomon, 1982; Solomon *et al.*, 2000). The two species are found in a wide range of habitats and oviposition occurs on various plants. *Anthocoris nemoralis* is mostly found on perennials, whereas *A. nemorum* is found on both perennials and annuals (Anderson, 1962a; Collyer, 1967; Fauvel, 1999; Sigsgaard, 2004, 2005).

Both anthocorids overwinter as adults making it possible for them to be active as soon as weather permits (Sigsgaard *et al.*, 2006). Predation in spring can delay or even hinder the build-up of pest infestations, a delay permitting time for the more specialist natural enemies to arrive in the field (Symondson *et al.* 2002). Understanding the life-cycle and distribution of anthocorids in time and space can help predicting and targeting conservation biocontrol efforts for improving biological control in orchards. Knowledge of prey preferences is of importance for predicting predation in the field. *A. nemorum* has been considered to be a prey generalist, but preferences for some greenhouse aphid species are known (Meyling *et al.*, 2003).

The purpose of the current study was a) to determine the life-cycle and number of generations of *A. nemorum* in Denmark, b) to assess the distribution of *A. nemorum* between apple trees and the orchard herbal vegetation and the distribution of the two anthocorids between apple and pear trees, c) to make a preliminary assessment of the potential

contribution by anthocorids to pest control based on field data, d) to assess prey preferences of the two anthocorid species to validate findings from field studies and assist in evaluating the potential of the two species for biological control purposes.

2. Materials and methods

In 2001 arthropods were sampled in three apple orchards on Zealand: an unsprayed orchard at Rørrendegaard in Tåstrup, belonging to the Royal Veterinary and Agricultural University, located 15 km east of Copenhagen, latitude 55.1^{o}N (20-25 m a.s.l); an organic orchard at Jørlunde, near Slangerup (55^{o}50'N, 12^{o}11'E; 10–20 m a.s.l.); and an organic orchard near Frederikssund (55^{o}52'N, 12^{o}5'E; 20–40 m a.s.l.). Samples were taken from May to October 2001. In 2002 the orchard at Rørrendegaard was again sampled. That same year, a pear orchard belonging to the Pometum, managed following integrated pest management practices, was also sampled. In 2002, samples were also taken in an IP-managed orchard on the island of Fejø, where the sampled pear and apple blocks were separated by a windbreak (54^{o}57'N, 11^{o}22'E; < 5 m a.s.l.). Samples were taken from late May/ June until September 2002. The orchard block where samples were collected was all between 0.5 and 1 ha.

The orchard at Rørrendegaard comprised a mixture of several apple varieties (2-4 trees of > 40 varieties, each variety; 15-20 y in age). It received no chemical input. The orchard in Jørlunde was 13-15 y old, and had been converted from conventional production 10 years earlier. This orchard had several varieties. Samples were taken in the Swedish variety, Elsa, which is an early variety. Trees in Frederikssund were four years old, all cv. Discovery. The orchard on Fejø included both apple and pear trees ca. 12 years old, and was managed conventionally. Pear trees were cv. Clara Friis, while apple trees were cv. Aroma. The pear

trees at the Pometum site were approximately 20 y old, and comprised a mixture of several pear varieties (2-3 trees of > 20 varieties).

Beating funnel samples from trees were collected by beating one branch on each of ten randomly selected trees with a stick. The stick was covered with cloth and rubber band to avoid damaging branches. The opening of the beating funnel measured 40 x 60 cm. While beating a branch the beating funnel was held under the branch. A plastic bag attached to the plastic pipe at the end of the funnel served to collect samples. Five to ten samples were taken in each orchard at each sampling date. The sweep net opening measured 36 cm in diameter. Sweep net samples were taken from annual vegetation between trees and –in the case of Rørrendegaard and the orchard on Fejø where vegetation under trees was cut very short – along the hedgerow at the edge of the orchard. No sweep net samples were taken in the Pometum, where grass was also kept very short, and no hedgerow was nearby. Each sample consisted of ten double-sweeps. Five to ten sweep net samples were taken in each orchard on each sampling date. In the case of beating funnel samples, arthropods were identified to order, family, genus or species, while for sweep net samples only anthocorids were identified. Anthocorids were further grouped as small (1^{st} -2^{nd} instar) medium (3^{rd} -4^{th} instar) and large nymphs with visible wing buds (5^{th} instar).

For the preference studies leaves of pear (cv. Clara Friis) and apple (cv. Prima) were field collected at Rørrendegaard immediately before the experiment. For experiments a-e, individual healthy and undamaged leaves were selected measuring 3-4 cm in length and 2-3 cm in width. In experiment f, larger mature leaves were used, and the leaftip, ca 1/4 the size of the lamina, was removed with a scalpel immediately prior to the experiment to fit them into experimental cups.

A. nemorum females were collected from organic orchards and adjacent habitats five to ten days prior to the experiments and were kept in thermo cabinets (L16:D8 photoperiod, 20 ±

1°C) until they were used in the assays. *A. nemoralis* were reared in thermo cabinets under the same light and temperature regime. The *A. nemoralis* culture originated from insects obtained from a laboratory rearing facility (EWH BioProduction). Anthocorids were kept in transparent plastic cages 7 cm in diameter and 8 cm high. Leaves of *Pilea peperomiodies* Diel (Urticaceae) were provided for oviposition and additional moisture. Twice a week, fresh leaves were provided and old leaves removed. Leaves with eggs were moved to new cages ensuring equal age cohorts in individual cages. At the same times *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) eggs were provided. A maximum of ten large nymphs or adult anthocorids were kept in each cage. In all experiments field collected *A. nemorum* of unknown age were used. In the case of *A. nemoralis* one week old individuals were used.

Cacopsylla pyri (L.) (Hemiptera: Psyllidae) was collected from a greenhouse rearing established a few months earlier. Apple branches with eggs and young nymphs of the apple psyllid, *Cacopsylla mali* Schmidberger, were collected from the field and kept in the greenhouse prior to the experiments. Green apple aphids, *Aphis pomi* De Geer (Hemiptera: Aphididae) were obtained from a laboratory culture established from field collected individuals a month prior to experiments and reared on pear leaves. For the preference experiment comparing two apple aphids, 4th instar *A. pomi* and the rosy apple aphid, *Dysaphis plantaginea* (Passerini) were collected from unsprayed apple trees immediately before the experiment; as much as possible, aphids of a uniform body size were used. Fruit tree red spider mites, *Panonychus ulmi* (Koch) (Acari: Tetranychidae), were also collected from the field.

Preference was assessed in small units (30 ml plastic cups). To provide water and support for leaves the base of the cages were covered with 3% agar, into which the petiole was inserted. Pear leaves were used in experiments a-c, while apple leaves were used in experiments d-f (experiments defined more fully below). Fifteen individuals of each prey

were used in the preference experiments, except in the experiment with *C. mali* where 20 individuals were used, and in experiment e) where ten individuals of each prey were used. Preys were allowed to settle for at least 15-20 min. before a predator was introduced into the assay chamber. Experimental durations were 1h, 1.5 h or 3h, adjusted after prey consumption. Preferences were assessed in terms of the number of prey eaten and biomass consumed. To calculate the biomass consumed by *A. nemorum* and *A. nemoralis* in the experiments, groups of 20 prey individuals were dried for 48 h, and subsequently weighted on a Mettler AE240 analytical balance, with an accuracy of 10 µg. The mean weight for groups was calculated, and subsequently divided by 20 to calculate individual dry weights. Anthocorids were starved for 24h prior to experiments. Predator-free cages (controls) were used to assess prey mortality in the absence of predators.

Preference experiments included: a) the two anthocorids preference for 1-2nd instar *C. pyri* or *A. pomi* (3h) (both n = 15 females; control n = 6). b) Preference by *A. nemoralis* for female *P. ulmi* or 1-2nd instar *C. pyri* (1h) (n = 19 [13 males and 6 females]; control n = 9). This experiment was later repeated with c) half of the *A. nemoralis* having had 24-h exposure to surplus *P. ulmi* prior to the 24-h starvation period (both n = 12, with 5 females in the treatment with prior exposure and 4 in the non-exposed; control n = 5). d) Preference of *A. nemorum* for 1-2nd instar *C. mali* or female *P. ulmi* with 20 of each prey (3h) (n = 9 [6 females and 3 males]; control n = 4). e) Stage-specific preference of *A. nemoralis* for eggs or young 1-2nd instar nymphs of *C. pyri* (1h) (n = 11). f) A comparison of *A. nemorum* preference for *A. pomi* or *D. plantaginea* (1.5 h) (n = 17). This final experiment was assessed after 1.5 and again after 2, 3, 4, 5 and 12 h, to assess whether experimental duration affected preference, with possible implications for interpretation.

Predation behaviour of the two anthocorid species towards *C. pyri* was observed in cages measuring 30 × 30 × 60 cm (*A. nemorum*: n = 15, *A. nemoralis*: n = 13). Cages were

transparent with a netted sliding door of 30 × 60 cm. In each cage a fresh-cut pear twig with three fully developed leaves was placed. Securing the twig with the basal part through a hole in the lid of a water-filled jar provided support and water. The twig was infested with 20 4th instar *C. pyri*. By the onset of the experiment one 24-h starved adult female anthocorid was carefully released onto the twig. Each anthocorid was observed for at least one hour. After an hour any ongoing feeding was observed until it ended to assess the full duration of feeding. Observations ceased if an anthocorid left the plant. During the observations behaviours recorded were a) insect resting or grooming, b) insect walking, c) encountering prey, defined as physical contact, d) attacking prey, and e) feeding on prey. Further the anthocorids position on the plant was recorded with respect to height (low, mid or top leaf) and with respect to plant structure (stem, leaf axis, dorsal side of leaf, ventral side of leaf). Time was registered in seconds with a stopwatch. In addition, the number of psyllids leaving the plant during the observation period was registered.

Data were analysed with ANOVA using SAS/STAT software (SAS Inst., 2003). If necessary data were transformed using the transformation best meeting requirements for normal distribution and homogeneity of variances. If an appropriate transformation was not possible, data were analyzed by nonparametric analysis. Orchard data were first examined for species abundance and associations between prey and predators. A lagged correlation analysis of *A. nemorum* effect on *C. mali* densities was carried out using PROC CORR (SAS Inst., 2003; Van Driesche, 1983).

Preference data were corrected with Abbotts mortality factor (Abbott, 1925) for mortality in controls. Prey preferences were analysed by calculating Manly's Alfa index of prey preference (Manly et al. 1972). The index was calculated by the following formula, which takes food depletion into account:

$$\alpha_i = \frac{\ln((n_{i0} - r_i)/n_{i0})}{\sum_{j=1}^{m} \ln((n_{j0} - r_j)/n_{j0})}, i = 1, \ldots, m \quad (1)$$

Where α_i is the preference for prey type 1; ni_0 and nj_0 are the number of prey type 1 and prey type 2, respectively, present at the beginning of an experiment; and r_i and r_j are the number of prey type 1 and prey type 2, respectively, remaining after experimentation. Thus, the value of α_i will fall between 0 and 1, with values larger than 0.5 indicating a preference. Manly's indices were compared between prey types using a two-tailed paired *t*-test (SAS Inst., 2003). In addition to the calculation of preference index, transformed mortality data and biomass consumption were analysed by ANOVA (SAS Inst., 2003) to test for differences in 1) the number of prey killed in the prey-combinations and 2) the numbers killed and biomass consumed in the two prey combinations. Data on mortality and biomass consumption were analyzed using Proc Mixed (SAS Inst., 2003). For models assuming normal distribution they were transformed using arcsine (√ (β/total prey density) prior to ANOVA analysis (Bibby *et al.*, 2004). Significant interactions were further analyzed using the slice option of the Proc Mixed (SAS Inst., 2003). The preference experiment f), where preference was assessed with intervals on each replicate, the repeated measurements function in Proc Mixed (SAS Inst. 2003) was used.

3. Results

3.1 Anthocorids in apple and pear trees

Females of *A. nemorum* were observed to be active on apple beginning with the 3 May sample in 2001 (Fig. 1). The first males were seen in mid-June, indicating that the offspring of the overwintering females had begun to reach the adult stage. The average number of *A. nemorum* in beating samples was (mean ± SE) 1.35 ± 0.09 individuals per sample across the season in the three apple orchards (Fig. 1). Densities of *A. nemoralis* in apple were low, with only 0.09 ± 0.03 per sample. In 2002 densities of *A. nemorum* and *A. nemoralis* in apple at Rørrendegaard were similar to those in 2001, with an average of 1.89 ± 0.09 and 0.09 ± 0.04 individuals per sample, respectively. In the two abutting orchards on Fejø, the average densities in apple were 0.27 ± 0.10 *A. nemorum* and 0.07 ± 0.05 *A. nemoralis* per sample whereas there were 0.04 ± 0.03 *A. nemorum* and 7.4 ± 1.5 *A. nemoralis* per sample in the adjacent pear block. Densities of *A. nemoralis* increased sharply through August apparently in response to a severe infestation of psyllids (Fig. 2). Very few anthocorids were recovered from the pear trees of the Pometum (*A. nemorum* 0.07 ± 0.04, *A. nemoralis* 0.02 ± 0.02). *A. nemorum* and *A. nemoralis* were the dominant anthocorids both years. Only ten other Anthocoridae specimens were encountered.

3.2 Generations of A. nemorum

In two of the tree orchards sampled in 2001 there were two distinct generations of *A. nemorum* (Fig. 1b, c). In the third orchard, Rørrendegaard, where the density of *A. nemorum* was highest, the distinction between generations was less clear, probably because of an overlap between generations (Fig. 1a). Data from 2002 in Rørrendegaard again suggest that two generations occurred (Fig. 1d), while data from Fejø are not sufficient for an assessment of the number of generations.

3.3 Dynamics of anthocorids with psyllids and aphids

At Rørrendegaard, *C. mali* were abundant (Fig. 3). As *A. nemorum* preys upon eggs and nymphs but not adults of *C. mali* (Sigsgaard *et al.*, 2006) the strongest correlation between numbers of *C. mali* and *A. nemorum* was found for the log-transformed numbers of adult psyllids one week following the *A. nemorum* sample. These data would reflect any effect the *A. nemorum* had had on nymphs ($R^2 = 0.89$, $F_{1,10} = 79.9$, $P < 0.0001$). *C. mali* numbers also correlated with *A. nemorum* numbers in the other two orchards (Frederikssund: $R^2 = 0.52$, $F_{1,9} = 8.5$, $P < 0.02$, Jørlunde: $R^2 = 0.55$, $F_{1,9} = 10.9$, $P < 0.009$). An analysis across orchards revealed no significant effect of orchard or *A. nemorum* × orchard, allowing for a model across orchards: *A. nemorum* = -0.62 + 5.69 × log10 (*C. mali* after 7 days) ($R^2 = 0.81$, $F_{1,31} = 130.6$, $P < 0.0001$). In 2002 apple psyllids were again common in Rørrendegaard (26.91 ± 3.57) and a similar correlation for 2002 was significant ($R^2 = 0.72$, $F_{1,6} = 15.7$, $P < 0.007$). Their densities on Fejø were low (0.03 ± 0.03).

Aphids can be a serious pest in apple, particular *A. pomi* and *D. plantaginea* are of concern but aphid densities in the two years were low, so a similar regression analysis as for *C. mali* and *A. nemorum* was not possible.

In the pear orchard on Fejø in 2002 the primary pest was *C. pyri* (85.69 ± 9.27) (total sampled 3455). The log-transformed numbers of adult *C. pyri* one week following the *A. nemoralis* sample was near significant ($R^2 = 0.86$, $F_{1,3} = 12.6$, $P = 0.07$) (Fig. 2). In contrast to Fejø, the *C. pyri* average sample was only 0.47 ± 0.13 at the Pometum. Across the two year period an additional 91 psyllids, which were not identified to species, were sampled in pear.

3.4 Anthocorids in the annual vegetation

Samples from the annual vegetation were taken to assess to what extent anthocorids utilize this habitat in the orchards, and whether the use of this habitat is constant or changing over the season. The two sampling methods used were different, thus excluding direct comparison

of densities. However, the relative densities of *A. nemorum* were higher in the trees in spring and early summer, while from mid-July to late August *A. nemorum* was relatively more numerous in the annual vegetation. After mid-end August, anthocorid densities in trees increased again (Fig. 4). Especially in two of the orchards (Rørrendegaard and Jørlunde) stinging nettle (*Urtica dioica* L. (Urticaceae)) was common in the annual vegetation, and *A. nemorum* was often observed in this weed. A similar pattern with more *A. nemorum* found in the annual vegetation in mid-summer was observed in 2002 in the orchard at Rørrendegaard. In Fejø the number of *A. nemorum* was too low for an interpretation of generations or seasonal movements. No *A. nemoralis* were found in the annual vegetation in 2001 and only few in 2002 (Rørrendegaard, 0.17 ± 0.08, Fejø: 0.19 ± 0.08).

3.5 Preference

The average individual weights of prey used in choice experiments, in cases where prey sizes were not equivalent, were: *C. pyri* eggs (0.008 ± 0.004 mg, n = 10), *C. pyri* 1-2 instar (0.020 ± 0.003 mg, n = 6), *C. pyri* 2-3 instar (0.032 ± 0.011 mg, n = 10), *A. pomi* 1-2 instar (0.041 ± 0.017 mg, n = 10), *P. ulmi* females (0.012 ± 0.003 mg, n = 10).

Though psyllids (*C. pyri*) are the main pests in pear, they often co-occur with aphids. *A. pomi* may attack both apple and pear. In apple, aphid infestation (*A. pomi* and/or *D. plantaginea*) can be severe. Psyllids in apple may be numerous, but rarely cause considerable economic loss. In preference experiment a) there was no control mortality. In the case of *A. nemoralis*, the *A. pomi* dead by the conclusion of the experiment (0.8 ± 0.3) were all dead without signs of feeding. In contrast, 1.2 ± 0.3 2nd instar *C. pyri* were eaten by *A. nemoralis*, and no dead *C. pyri* without signs of feeding were observed (Fig. 5a). *A. nemorum* killed (dead and eaten) more *A. pomi* (1.3 ± 0.3 and 0.7 ± 0.2) than *C. pyri* (0.1 ± 0.1 and 0.6 ± 0.2) (Fig. 5a). In terms of prey killed, *A. nemoralis* preference for *C. pyri* over *A. pomi* was α =

0.59 ± 0.12 (c.i. 0.32-0.86), including α = 0.5 for no preference. For *A. nemorum* α = 0.27 ± 0.08 (c.i. 0.10-0.43), indicating a preference for the aphid prey over the psyllid. Preference indices of the two anthocorid species differed significantly (t = 2.30, df = 25, p = 0.03). There was a crossed effect of anthocorid species × prey species on number of prey killed (Proc Mixed, $F_{1,28}$ = 10.67, p = 0.003), A slice-analysis showed that *A. nemorum* killed significantly more *A. pomi* than *C. pyri* ($F_{1,28}$ = 12.4, p = 0.002), and killed more *A. pomi* than *A. nemoralis* ($F_{1,53.7}$ = 8.2, p = 0.006). An analysis restricted to prey with signs of feeding also shows a crossed effect of anthocorid species × prey species ($F_{1,56}$ = 7.8, p = 0.007). A slice analysis shows that significantly more *C. pyri* than *A. pomi* were eaten by *A. nemoralis* ($F_{1,56}$ = 14.0, p = 0.0004). No significant difference in prey eaten is found for *A. nemorum* ($F_{1,56}$ = 0.04, p = 0.86). *A. nemorum* ate significantly more *A. pomi* than *A. nemoralis* ($F_{1,56}$ = 4.3, P = 0.04). In terms of biomass consumed, *A. nemoralis* ate an average of 0.04 mg *C. pyri*, while the slightly larger *A. nemorum* ate an average of 0.02 mg *C. pyri* and 0.03 mg *A. pomi*, Since the weight of the two prey types in this trial are equivalent, an analysis on biomass consumed yields the same conclusions as for number of prey eaten.

Anthocoris nemoralis preferred *C. pyri* nymphs (3.5 ± 0.4 killed) to female *P. ulmi* (0.6 ± 0.2) (Fig. 5b). Manley's α was 0.95 ± 0.03 (c.i. 0.89-1.00), and the number of *C. pyri* killed was significantly higher than that of *P. ulmi*, with a significant effect of prey (Proc Mixed, $F_{1,17}$ = 68.2, p < 0.0001), but no significant effect of prey x sex (Proc Mixed, $F_{1,17}$ = 3.7, df = 15, p = 0.07). In terms of biomass consumed the preference for *C. pyri* was more pronounced, as nymphs were approximately four times heavier than the spider mites. One *C. pyri* died in the 9 controls. Dead *P. ulmi* had no clear signs of feeding, but feeding could have occurred, thus a comparison restricted to prey killed was most correct. The same was the case in the following two preference experiments.

Adding surplus spider mites to *A. nemoralis*' diet for 24h before the 24h starvation period did not lead to an increase in predation rates. Few mites were killed (Fig. 5c) and, the effect of experience was not significant (Proc Mixed, $F_{1,19} = 2.31$, $p = 0.15$). There was no significant effect of sex. Three *P. ulmi* died in the 5 controls.

Like *A. nemoralis*, *A. nemorum* preferred psyllids, in this case *C. mali* to *P. ulmi*, though its preference was less pronounced, with Manly's $\alpha = 0.64 \pm 0.05$ (c.i. 0.52-0.77). It killed an average of 4.1 ± 0.7 female fruit tree spider mites and 7.9 ± 1.3 *C. mali* (Fig. 5d), with a significant effect of prey ($F_{1,22} = 22.3$, $p < 0.005$), but no significant effect of sex or sex × prey. One *C. mali* died in the 4 controls.

A. nemoralis killed significantly more *C. pyri* eggs (5.3 ±1.1) than nymphs (2.6 ±0.6) (Fig. 5e), $\alpha = 0.72 \pm 0.07$ (c.i. 0.57-0.89) ($t = 4.49$, $df = 20$, $p = 0.0002$). There was no significant effect of sex, but the reduced model was significant for the effect of prey instar on prey killed ($F_{1,20} = 4.8$, $p = 0.04$). There was no significant difference between the biomass consumed of nymphs and eggs ($F_{1,20} = 1.2$, $p = 0.30$).

The experiment assessing preference of *A. nemorum* for *A. pomi* or *D. plantaginea* after 1.5 h, found 1.1 ± 0.2 *D. plantaginea* and 2.2 ± 0.1 *A. pomi* had been killed (Fig. 5f). The time factor slightly reduced the preference found for *A. pomi*, as more of these prey were removed than the less preferred *D. plantaginea*. After 1.5 h it was 0.69 ± 0.04 (c.i. 0.61-0.77) ($t = 2.2$, $df = 33$, $p = 0.03$), and by the final assessment after 12 h 0.66 (c.i. 0.60-0.72) ($t = 12.33$, $df = 32$, $p < 0.0001$). There was a significant crossed effect of prey × time ($F_{5,177} = 2.59$, $p = 0.02$), and highly significant main effects of prey and time ($F_{1,177} = 183.3$ and $F_{5,177} = 61.5$, $p < 0.0001$). There was no control mortality in this trial, and no aphids were observed dead but not eaten.

No significant difference was found in the two anthocorids' predation rate towards 4th instar pear psyllids (*A. nemorum*: 1.6 ± 0.2 nymphs, *A. nemoralis*: 1.7 ± 0.4 nymphs).

However, the success rate (prey eaten/encounters) (log-transformed) was significantly higher for *A. nemoralis* (0.7 ± 0.07) than for *A. nemorum* (0.5 ± 0.10) (Proc GLM, $F_{1,23} = 4.41$, $p < 0.05$). Further, the handling time for *A. nemoralis* (45.0 ± 8.0 min. spent to eat one prey) (log transformed for analysis) was double that found for *A. nemorum* (21.9 ± 5.4 min.) (Proc GLM, $F_{1,23} = 6.34$, $p < 0.02$). The more 'restless' behaviour of *A. nemorum* was also seen from the fact that nymphs left twigs with *A. nemorum* (2.4 ± 1.22 nymphs), while this was not observed with *A. nemoralis* (data log transformed for analysis) (Proc GLM, $F_{1,26} = 8.11$, $p < 0.009$). Both anthocorids spent most of the time resting on the leaves, with significant main effect for position ($F_{4,111} = 5.78$, $p < 0.0003$), but no significant effect of species or position × species.

4. Discussion

4.1 Orchards

Though the ages and localities of the orchards differed quite widely all had moderate or no input of pesticides and anthocorids were present from early in the season. The oldest and least managed orchard in Rørrendegaard maintained the highest arthropod densities, including highest anthocorid densities.

The early presence of anthocorids in apple and pear means that they can play an important role in the early natural control of insect pests. Generalists can be of key importance if they occur early in the population development of an insect pest, and at this early stage control or delay the population development (Symondson et al. 2002). Such a delay will provide better chances that the later occurring specialists can keep the pest attack on an acceptably low level.

Only on Rørrendegaard were densities of *C. mali* high. This psyllid infrequently reaches pest status, but can then reduce plant growth and vigour. The Danish advisory service only

observes sporadic cases. Few cases of outbreaks are published (Kagan & Lewartowski 1978). Overwintering apple psyllid eggs and hatching nymphs may play a positive role in sustaining the population of beneficials 'laying-in-wait' for later, more problematic, pests. In contract to *C. pyri*, which normally has three generations in Denmark, *C. mali* is univoltine, which may help to explain its low pest status. The lower densities of *C. mali* observed in July (Fig. 3) can be a result of the adults moving into shade or trees surrounding the orchard, later returning to the orchard for oviposition (Brittain, 1922, Lauterer, 1999). The correlation between apple psyllids and *A. nemorum* suggests that the apple psyllids can contribute to the diet of *A. nemorum*.

4.2 Anthocoris nemorum dominates in apple, but A. nemoralis in pear

Data from apple in 2001 and 2002 and from the two neighbouring apple and pear orchards in 2002 show that while *A. nemorum* is by far the most numerous in apple, *A. nemoralis* dominates in pear. However, according to Solomon (1982) *A. nemoralis* can be common in apple orchards in Europe when the apple psyllid, *C. mali*, is present.

Even on Fejø, where the apple orchard and the pear orchard were only separated by a single row of alder, densities of *A. nemoralis* were high in pear but low in apple and vice versa. Thus the spread of *A. nemoralis* into the neighbouring apple orchard appears to be limited in extent.

The high density of *A. nemorum* in annual vegetation during mid-summer shows that *A. nemorum* is able to exploit both layers of the vegetation. Rich herbal vegetation can help maintain or attract anthocorids in an orchard in periods where there is few prey in the trees. English studies show that cornflower and corn chamomile can serve to attract anthocorids (Fitzgerald & Solomon 2004). *A. nemorum* is attracted to odours emitted from stinging nettle *U. dioica* (Dwumfour, 1992). *A. nemorum* was often observed on nettle, which was frequent in the annual vegetation. Nettle often has high densities of prey, including the aphid

Microlophium carnosum (Buckt.) (Perrin, 1975). Its role as an alternate feeding site has been documented for another group of generalist predators, the coccinellids (Burgio *et al.*, 2004).

4.3 Two annual generation of A. nemorum

In Scotland, *A. nemorum* generally has a single generation a year (Hill, 1957), while the species is bivoltine in Southern England (Collyer, 1967) and may have up to three generations in parts of France (Herard and Chen, 1985). Based on these observations, *A. nemorum* was expected to be univoltine in Denmark. Instead, there were second or at least partial second generations of *A. nemorum* in both years of the study. Since both summers were warmer than usual, it is likely that in cooler summers only one generation is completed. With a view to global warming, the occurrence of two generations may become more usual in the future.

4.4 Prey preferences

Preference can be an indicator for the proportion of a given prey; a predator may remove, when present together with other species. In cases where other prey is present, predation may be diverted, depending on the prey preferences of predators.

C. pyri is perhaps the most serious pest in pear. While *A. nemoralis* preferred *C. pyri* to *A. pomi*, *A. nemorum* showed no preference to either in terms of prey killed, while in terms of prey with visible signs of feeding, it preferred *A. pomi*. This finding shows that *A. nemoralis* is superior to *A. nemorum* for control of *C. pyri*, as has also been found in augmentative experiments (Sigsgaard *et al.*, 2006).

P. ulmi is an important pest in both apple and pear. However, both anthocorids (particularly *A. nemoralis*) preferred psyllids to *P. ulmi*. Prior exposure to *P. ulmi* did not change this preference in *A. nemoralis*. Though preference for female *P. ulmi* was low,

predation on spider mites may be important in the absence of more attractive prey or with younger instars or eggs of spider mites (Solomon, 1982).

The stage specific preference of *A. nemoralis* for *C. pyri* eggs shows that this predator can exert some control already at this stage, and also that it readily feeds on immobile prey. However, in terms of biomass, it did not eat more eggs than nymphs, when offered the choice.

Though being the smaller of the two, *A. nemoralis* had a higher success rate with the large 4th instar *C. pyri*. The longer handling time of *A. nemoralis* may reflect a better use of prey by this predator (ref). The importance of *A. nemoralis* for *C. pyri* control (Solomon *et al.*, 2000) is supported by its performance in the behavioural study as well as by it preference for *C. pyri*.

On apple *A. pomi* and *D. plantaginea* are important pests, and can co-occur. The trial demonstrated that *A. nemorum* prefer *A. pomi*. Such a preference may be even more pronounced in the field, where the *D. plantaginea* is protected in more closed pseudo-galls, than *A. pomi*, but this would require testing under more natural conditions. The consistency of preference over time, demonstrates a certain robustness of the method.

Across preference experiments, the preference for *C. pyri* compared to other prey was pronounced in *A. nemoralis*. *A. nemorum* preferred aphids to psyllids and (like *A. nemoralis*) preferred psyllids to spider mites. Among aphids it preferred *A. pomi* to *D. plantaginea*. Though preferences demonstrated by *A. nemorum* were less pronounced than those of *A. nemoralis*, they could still have a real impact in pest control in a field situation, such as cases where *A. pomi* and *D. plantaginea* co-occur.

4.5 Habitat and preference –being in the right time at the right place

The predominance of *A. nemorum* in apple and *A. nemoralis* in pear is supported by their prey preferences. Laboratory experiments showing a preference of *A. nemorum* to oviposit in apple

leaves and of *A. nemoralis* to oviposit in pear leaves (Sigsgaard, 2004) are in agreement with these findings. One mechanism guiding anthocorids to their preferred habitat and prey can be olfactory cues, as documented for *A. nemoralis,* with olfactory cues from pear trees with a psyllid infestation (Drukker *et al.*, 1995; Scutareanu *et al.*, 1997; Scutareanu *et al.*, 1999). The high densities of *A. nemoralis* in the Fejø pear orchard late in 2002 (Fig. 2) accompanied a serious infestation of pear psyllid, and it is possible that the predators were attracted to volatiles associated with the infestation. *A. nemoralis* also prefers to deposit eggs on leaf surfaces coated with the honeydew of *C. pyri* compared to leaves free of honeydew (Sigsgaard, 2005). Since the oviposition site selected by overwintering adult anthocorids in spring will determine the distribution of the far more numerous –and less mobile- offspring, oviposition preference will have a major impact on their distribution, which would be reflected in field samplings.

Prey and plant preferences of the two anthocorids predict their contribution to biological control in orchards to be quite sharply divided between apple and pear. Such information can be used to guide functional biodiversity for conservation biological control. Thus improved cover crop habitats for instance allowing patches of *U. dioica* could be of importance for conserving *A. nemorum* in the orchard, while such cover crops would be of less importance for *A. nemoralis*. Finally, results indicate that in cases where augmentative releases of anthocorids in orchards would be relevant, *A. nemorum* should be used in apple and *A. nemoralis* in pear.

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Figure legends

Figure 1. Average seasonal numbers of *Anthocoris nemorum* in three apple orchards in 2001 and one orchard in 2002 estimated from beating samples. a) Rørrendegaard, b) Jørlunde and c) Frederikssund and d) Rørrendegaard in 2002. Males (●), Females (□), small nymphs (Δ), medium nymphs (x) and large nymphs (+). Both the orchards of Jørlunde and Frederikssund display a smaller second generation. For the orchard at Rørrendegaard, with the highest *A. nemorum* numbers this is less clear, possibly caused by an overlap between the two generations. The numbers of *A. nemoralis* in apple were too low to be included in the graph. Note that to improve readability the y-axis of the subfigures are not identical

Figure 2

Average catches of *Anthocoris nemoralis* (□) in pear on Fejø in 2002. The *Cacopsylla pyri* population development in pear is shown on the secondary axis (dotted line).

Figure 3.

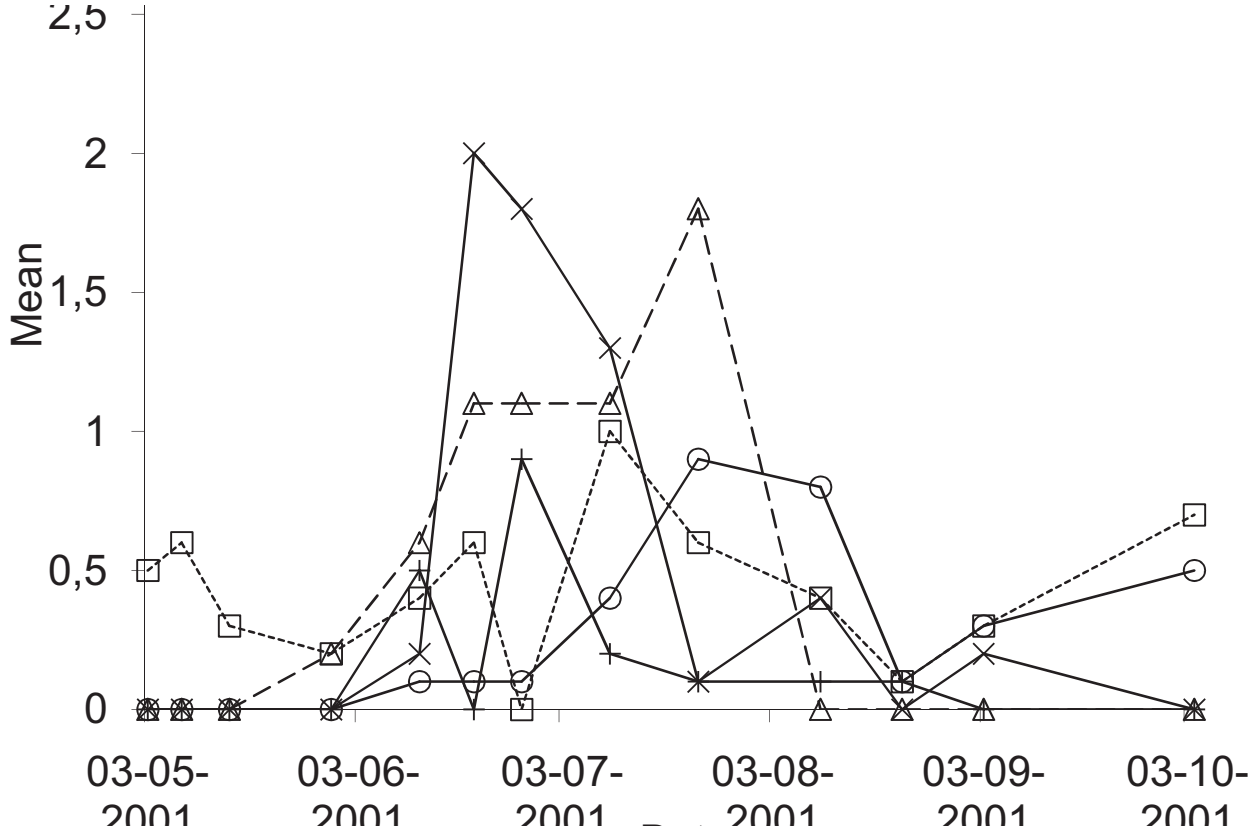
Seasonal occurrence of apple psyllids in the Rørrende orchard.

Figure 4.

Average seasonal samples (± SE) across a) three apple orchards in 2001 of *Anthocoris nemorum* and *A. nemoralis* from beating (trees) (+ and ×, respectively) and from sweep net (annual vegetation) (Δ and ○, respectively). Early and late season relatively more *A. nemorum* are sampled from trees, while mid-season relatively more are sampled from annual vegetation.

Figure 5.

Preference experiments with *A. nemorum* (grey) and *A. nemoralis* (white). Average number (mean ± SE) of a) 1-2nd instar *C. pyri* and *A.pomi* eaten and dead in (3h) (both n = 15 females, control n = 6). b) *A. nemoralis*' preference for female *P. ulmi* and 1-2nd instar *C. pyri* (1h) (n = 19, of which 13 males and 6 females, control n = 9). This experiment was later repeated with c) half the *A. nemoralis* having had 24-h exposure to surplus *P. ulmi* prior to the 24-h starvation period (both n = 12, of which 5 females in the prior exposure treatment and 4 in the non exposed, control = 5). In experiment a-c 15 prey of each type was provided. d) *A. nemorum's* preference for 1-2nd instar *C. mali* and female *P. ulmi* with 20 of each prey (3h) (n = 9, 6 females and 3 males, control n = 4). d) Stage-specific preference of *A. nemoralis* for eggs or 1-2nd instar nymphs of *C. pyri* (1h) (n = 11). e) *A. nemorum's* preference for *A. pomi* (▲), and *D. plantaginea* (○) (1.5 h) (n = 17, all females). This experiment was assessed after 1.5 and again after 2, 3, 4, 5 and 12 h.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2
1,5
1
0,5
0
Mean
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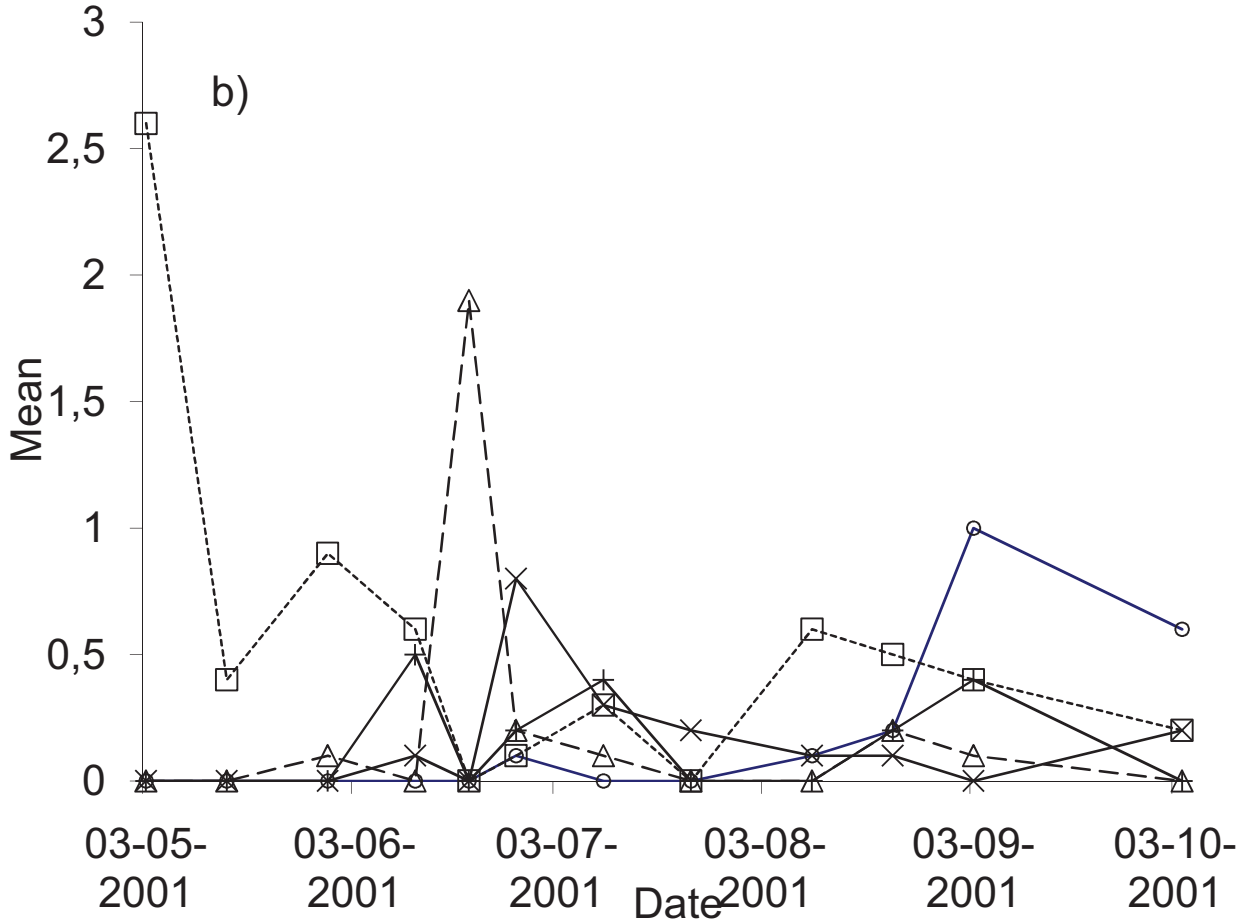
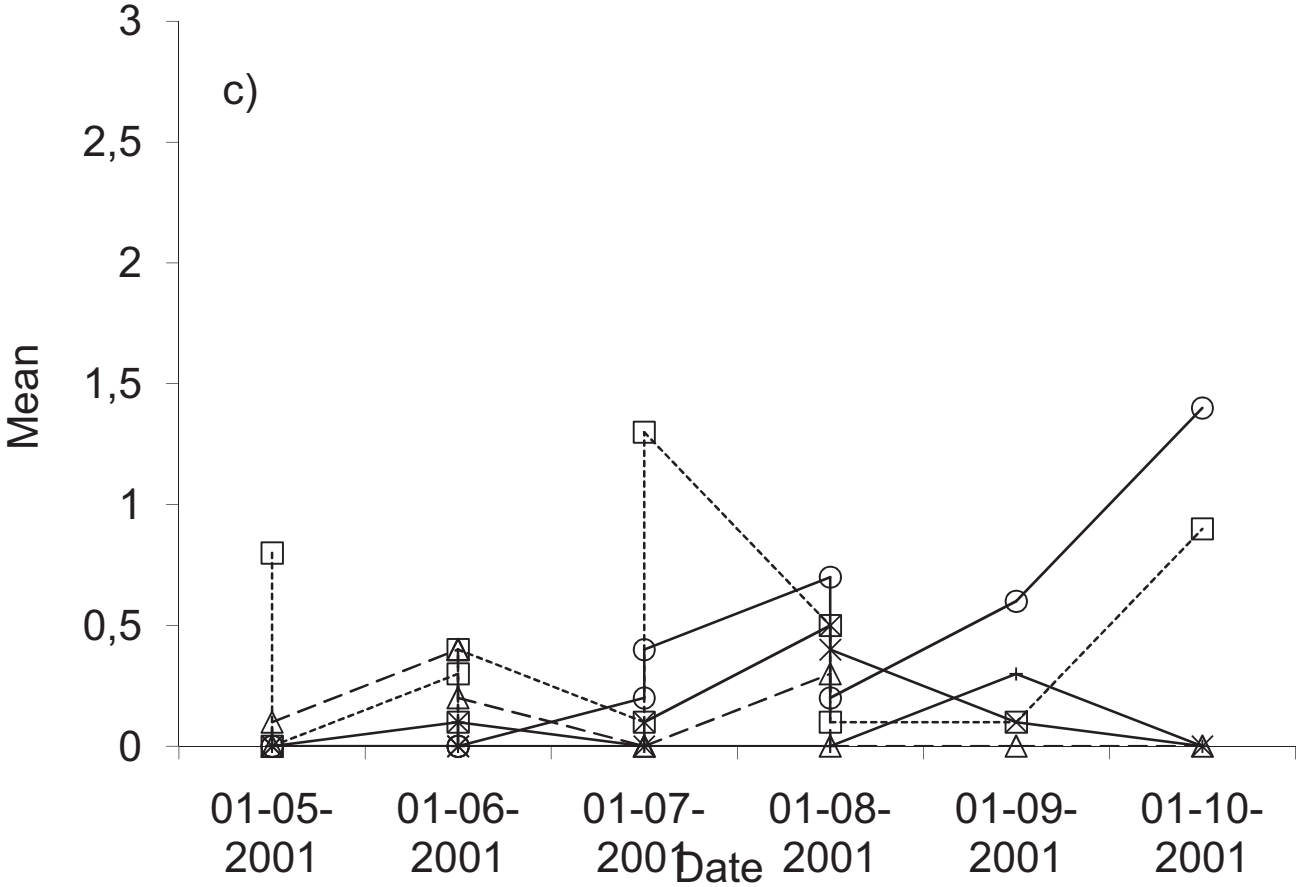
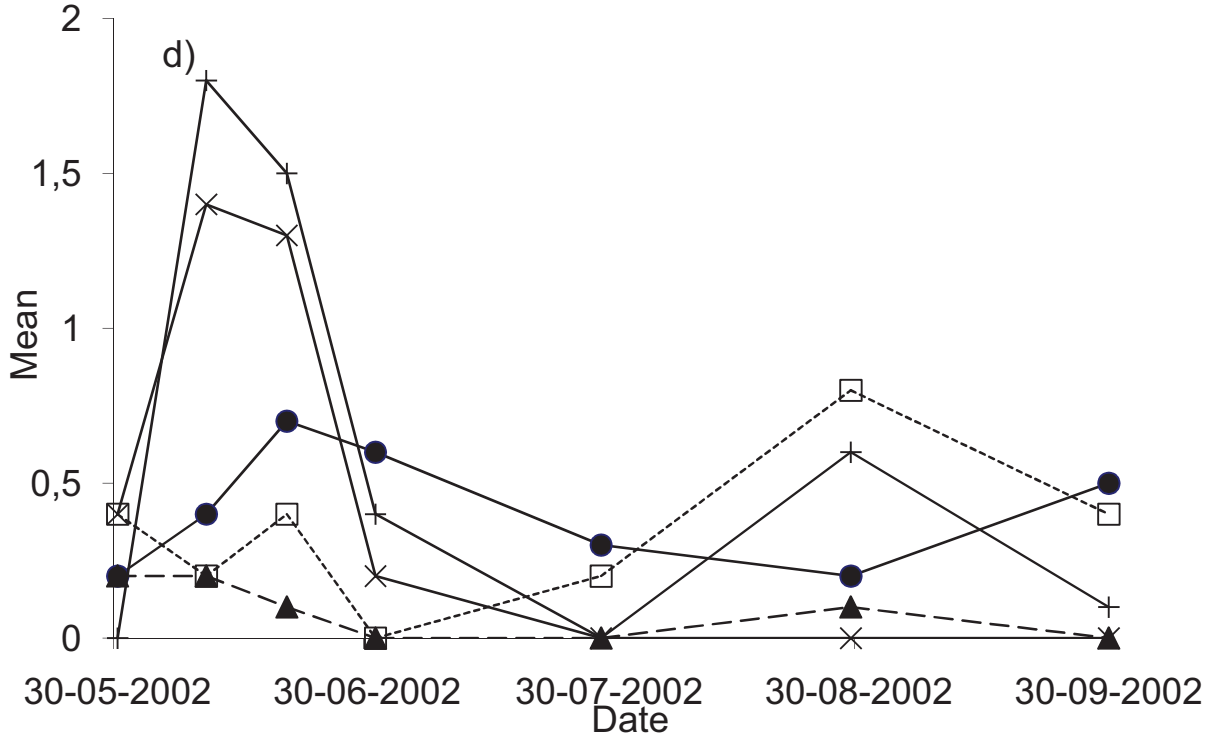
03-06-2001
03-07-2001
03-08-2001
03-09-2001
03-10-2001


b)
3
2,5
2
1,5
1
0,5
0
Mean
03-05-2001
03-06-2001
03-07-2001
03-08-2001
03-09-2001
03-10-2001
Date


c)
3
2,5
2
1,5
1
0,5
0
Mean
01-05-2001
01-06-2001
01-07-2001
01-08-2001
01-09-2001
01-10-2001
Date


d)
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1
0,5
0
Mean
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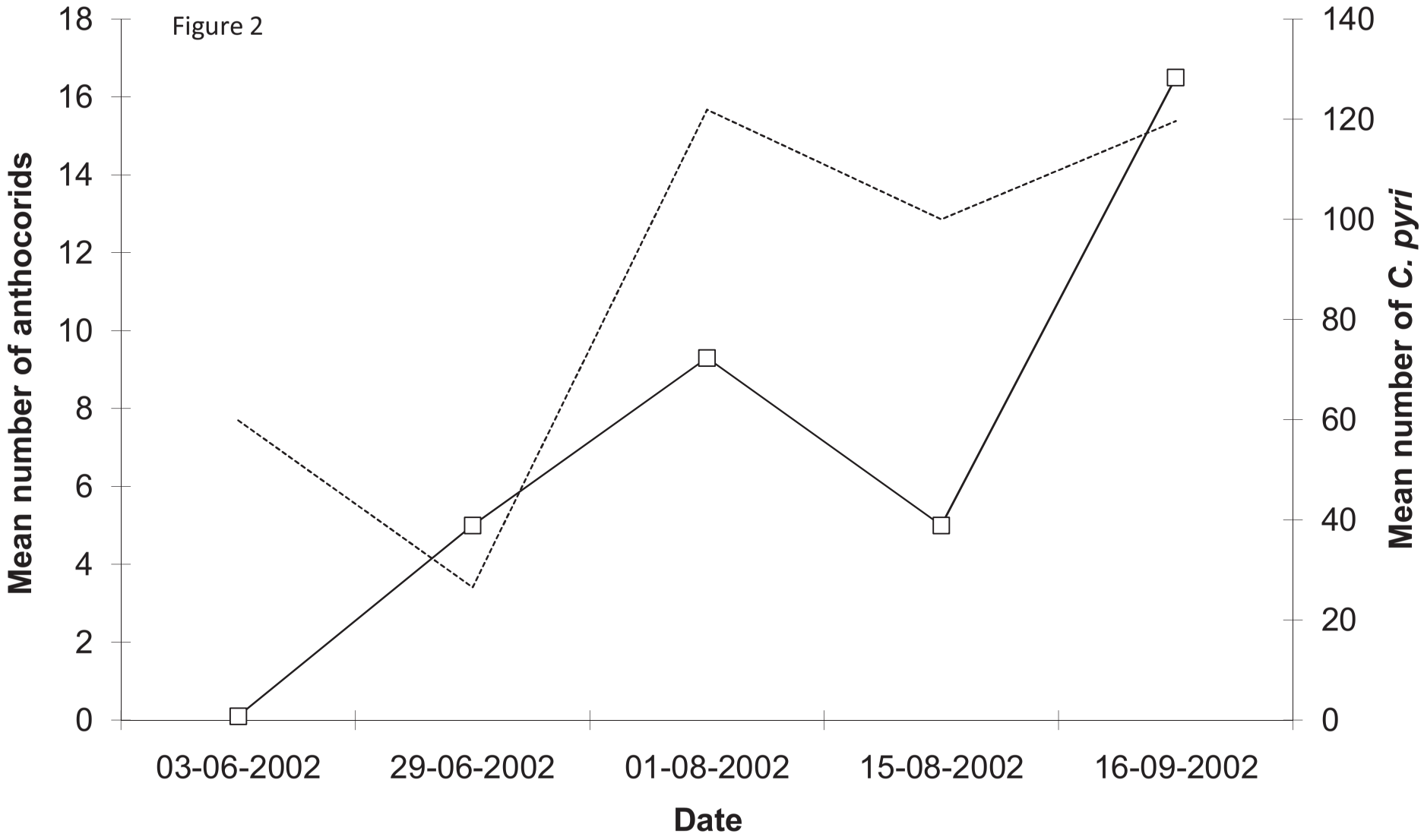
30-06-2002
30-07-2002
30-08-2002
30-09-2002
Date

Figure 2


Mean number of anthocorids
Mean number of C. pyri
Date
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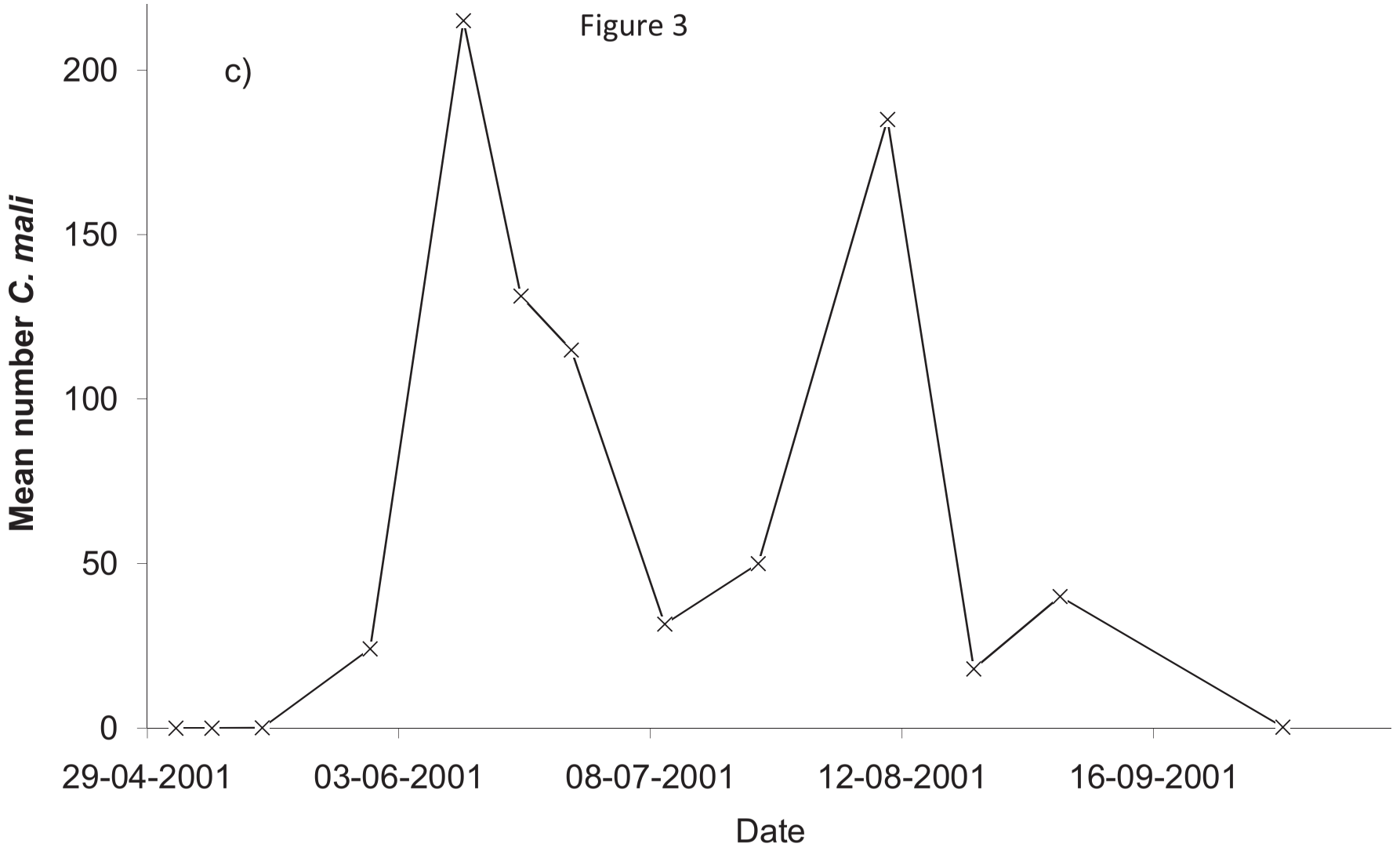
2
4
6
8
10
12
14
16
18
0
20
40
60
80
100
120
140
03-06-2002
29-06-2002
01-08-2002
15-08-2002
16-09-2002

Figure 3


c)
Mean number C. mali
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150
100
50
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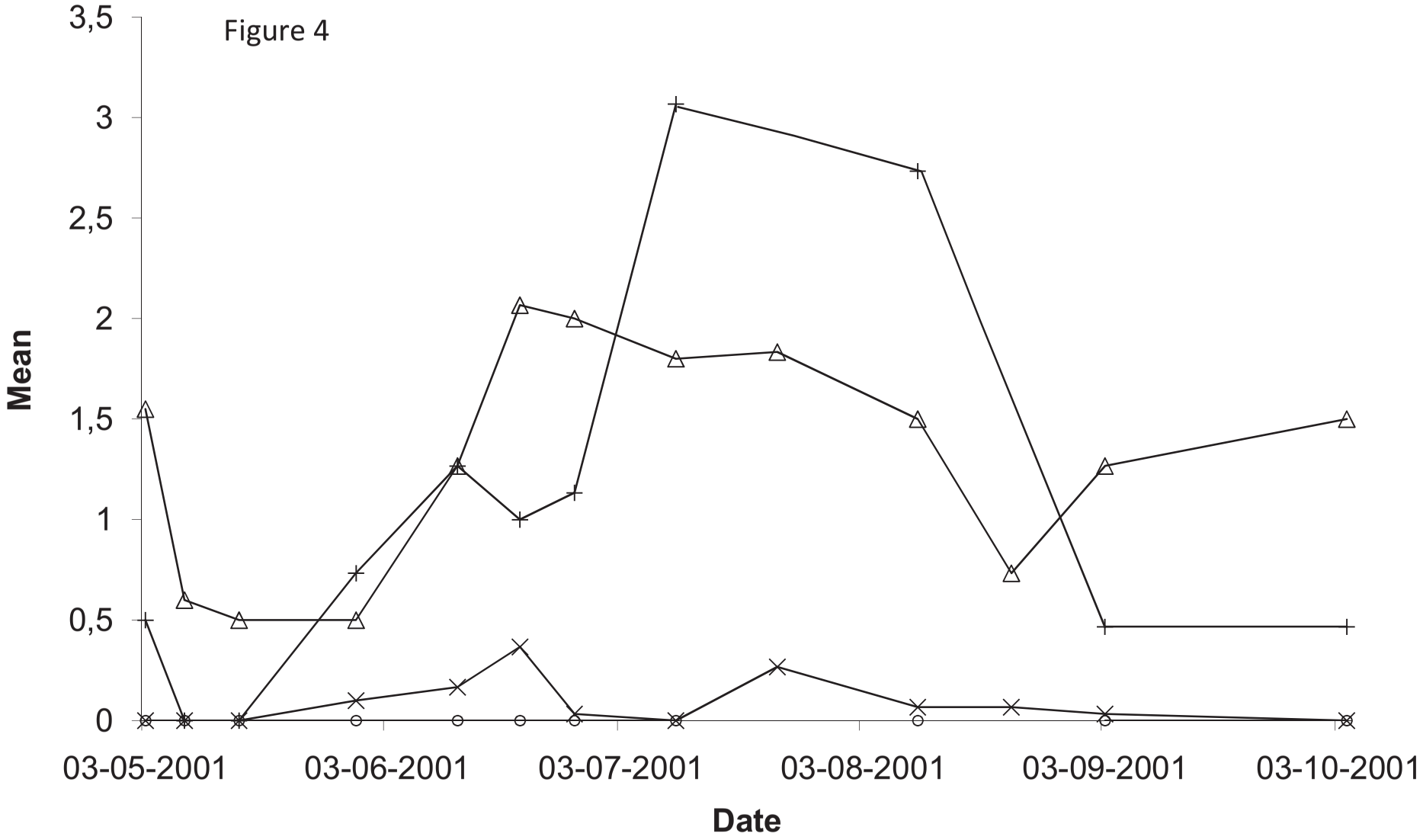
16-09-2001
Date

Figure 4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2
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Mean
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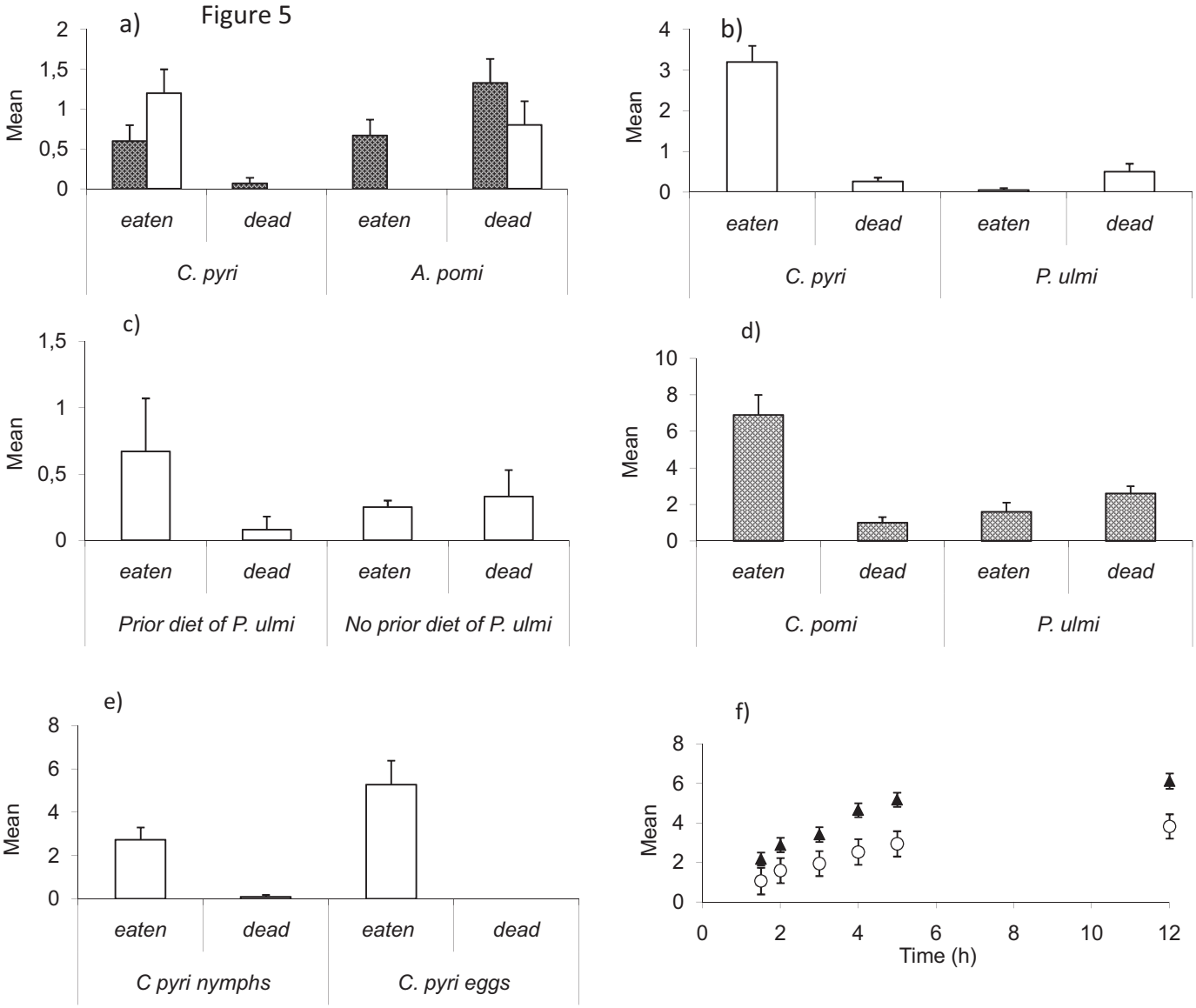
03-10-2001
Date

Figure 5


a)
Mean
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1,5
1
0,5
0
eaten
dead
eaten
dead
C. pyri
A. pomi
b)
Mean
4
3
2
1
0
eaten
dead
eaten
dead
C. pyri
P. ulmi
c)
Mean
1,5
1
0,5
0
eaten
dead
eaten
dead
Prior diet of P. ulmi
No prior diet of P. ulmi
d)
Mean
10
8
6
4
2
0
eaten
dead
eaten
dead
C. pomi
P. ulmi
e)
Mean
8
6
4
2
0
eaten
dead
eaten
dead
C pyri nymphs
C. pyri eggs
f)
Mean
8
6
4
2
0
0
2
4
6
8
10
12
Time (h)